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ATTENUATION OF ER:YAG LASER THROUGH HUMAN AND PORCINE LENS MICROSECTIONS MEASURED BY MEANS OF INFRARED SPECTROPHOTOMETRY

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Purpose To measure the absorption coefficient α, expressed in cm^{-1}, of a 2940 nm infrared radiation through microsections of human and porcine lenses.
Methods Human lens nuclei from extracapsularly cataract procedures and porcine lens from freshly enucleated porcine eyes were cryocut in 10, 15 and 30 µm sections. The cryosections were placed on microscope slides and covered by cover slips, whose edges were painted with uncoloured nail varnish to reduce bioliogical water evaporation.
The slides were placed into the microbeam of a Fourier Transform Infrared Spectrophotometer Nicolet 20 SXC to measure the variations of tissue absorbance versus wavelength between 1750 and 4750 nm. The spectrum obtained varies in shape and intensity according to the samples hydration degree. A mathematical approach using biological water and dry lens material spectra allows the measurement of tissue absorbance at 2940 nm according to the samples hydration degree.
The absorption coefficient α is obtained dividing absorbance value at 2940 nm by microsection thickness expressed in centimeter.
Results Absorption spectrum of dry tissue shows 5 peaks at 3049, 3249, 3377, 3408 and 3478 nm with a maximum at 3049 nm. Human absorption coefficient at 2940 nm varies from 344 ± 127 cm^{-1} in dry lens nuclei to 636 ± 236 cm^{-1} in fully hydrated samples. Porcine coefficient varies from 698 ± 143 cm^{-1} (dry tissue) to 1291 ± 265 cm^{-1} (hydrated samples).
Conclusions At 2940 nm, the lens absorption coefficient increases with tissue water content and makes Er:YAG laser particularly useful in cataract surgery.
Key-words Human nuclei, porcine lens, infrared spectrophotometry, absorption coefficient, Er:YAG laser, cataract surgery.

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CALCIUM DISTRIBUTION IN THE HUMAN LENS.

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Purpose. Calcium (Ca^{2+}) plays an important role in the normal physiology of lenses and disturbance of its homeostasis leads to development of cataract. The aim of this study was to localize Ca^{2+} in clear and opaque human lenses at the ultrastructural level and the lenses were investigated regarding the presence of loosely bound Ca^{2+}.
Methods. Human donor lenses were obtained from the Corneabank, Amsterdam. Clear lenses and lenses with small, equatorial opacities were processed according to the oxalate pyroantimonate technique (OPA).
Results. In control lenses small amounts of Ca^{2+} were found in the endoplasmic reticulum, nuclear envelope, Golgi fields and mitochondria of the epithelial cells and superficial fibers. From the superficial fibers to the intermediate cortical fibers the amount of Ca^{2+} precipitates along the membranes is increasing. In contrast to the control lenses large amounts of Ca^{2+} were observed in the cytoplasm of superficial and deep cortical fibers of opaque lenses. In addition small cytoplasmic and large extracellular vacuoles and stacks of myelin-like structures were found in opaque lenses containing large amounts of Ca^{2+}.
Conclusion. The observations reveal a significant difference in amount and localization of Ca^{2+} between clear lenses and lenses with small opacities. This may indicate a disturbance in the physiological homeostasis of Ca^{2+} which may be responsible for the local opacification.

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CHARACTERISATION OF THE SUBTYPES OF MUSCARINIC RECEPTORS INVOLVED IN THE ELECTROPHYSIOLOGICAL RESPONSE OF THE LENS TO ACETYLCHOLINE.

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Purpose To characterise the subtypes of muscarinic receptors involved in the electrophysiological response of the isolated lens to acetylcholine.
Methods The membrane potential of freshly-dissected lenses from a number of species was measured using the two intralenticular electrode technique.
Results Acetylcholine induces a reversible and dose-dependent membrane potential depolarisation of rat, rabbit, frog and human lenses. Furthermore, the stimulation with successive short pulse of acetylcholine does not cause any significant desensitisation in rat and rabbit. This characteristic allowed a pharmacological study of the acetylcholine response. In the rat lens, the response to short pulses of acetylcholine was totally inhibited by 100 nM 4-DAMP or pirenzepine. This indicates that the response is mediated by an M1 muscarinic receptor. The response to acetylcholine of the rabbit lens is totally inhibited by 100 nM 4-DAMP but not by 1 µM methoctramine or pirenzepine. Therefore the response appears to be mediated by an M3 muscarinic receptor. The sensitivity of the lens to acetylcholine appears to vary. The half maximal acetylcholine concentration is 4 µM in the rat lens and 0.2 µM in the rabbit lens.
Conclusions Although in a variety of species the lens is sensitive to acetylcholine, the response mechanisms involved differ. The rabbit lens responds to acetylcholine via M3 muscarinic receptors while the rat lens responds via M1 muscarinic receptors with a lower sensitivity.

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CLASSIFICATION OF CP49 AND FILENSIN: TWO LENS SPECIFIC INTERMEDIATE FILAMENT PROTEINS

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Purpose: To test whether CP49 and filensin represent a new class of intermediate filament (IF) proteins by determining their intron positions. Amino acid sequence data has shown that CP49 and filensin are homologous to other IF proteins but the overall percentage amino acid identity of these proteins is low when compared to other IF proteins. The intron positions are highly conserved within each class of IF protein and therefore the identification of the intron positions in CP49 and filensin would establish whether these proteins represent a new class of IF protein or if they should be included in existing groups (type I-V). The classification of new IF proteins such as CP49 and filensin will contribute to our understanding of the evolutionary origins of intermediate filament proteins.
Methods: Using CP49 and filensin intron sequences, specific primers were used to isolate P1 clones. The P1 phage system was used as large fragments (85-100kb) of genomic DNA can be cloned which will include the complete gene of interest. The P1 clones were sequenced to identify intron positions.
Results: The initial positional intron analysis has shown that CP49 and filensin can both be classified into the existing IF groups.
Conclusion: CP49 and filensin do not represent a new class of IF proteins and have evolved from a common ancestral gene. The isolation of the P1 clones has not only allowed investigation of the gene structure but will also enable examination of the regulatory sequences for these genes.
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